Pollinating Bees (Hymenoptera: Apiformes) of U.S. Alfalfa Compared for Rates of Pod and Seed Set

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ABSTRACT Alfalfa (=lucerne) flowers require visiting bees to trip the sexual column, thereby providing pollination and subsequent pod and seed set. Previous studies have compared the pollination values of different bee species solely by the speed with which they handle flowers and the proportion of visited flowers tripped. In this greenhouse study, five species of bees, including the three commercially managed U.S alfalfa pollinators, are likewise compared for their floral tripping frequencies. These bee species are also compared for the pod set and mature seed that results from their single visits to virgin flowers. Regardless of the identity of the pollinating bee, tripped flowers had the same probabilities of pod set and seed set. Thus, differences in the single-visit pollination efficiencies of the various bee species are entirely attributable to the proportion of visited flowers that they trip. Females of the alkali bee, *Nomia melanderi* Cockerell, and the alfalfa leafcutting bee, *Megachile rotundata* F, tripped 81 and 78% of visited flowers, respectively. Males of these species are significantly less effective (61 and 51%, respectively), but still significantly superior to the honey bee, *Apis mellifera* L. (22% of visited flowers tripped). These relationships are supported by field data for tripping frequencies. One candidate pollinator, *Osmia sanrafaelae* Parker, shows promise (44% tripped), but not the congeneric *O. aglaia* Sandhouse (13% tripped).

KEY WORDS *Megachile*, *Nomia*, *Apis*, *Osmia*, lucerne, pollination efficiency

THE MOST VALUABLE U.S. crop requiring bees for pollination is alfalfa (lucerne, *Medicago sativa* L.) (USDA-National Agricultural Statistical Service 1999). The nine million hectares of alfalfa hay grown in the United States feeds the dairy herds of the upper Midwest and western states. Alfalfa is also an important forage crop in many other countries outside of the tropics. Alfalfa seed production is centered in arid regions of the Pacific Northwest, the Intermountain West, and the Central Valley of California. In 1998, the six primary producing states of the United States (Pacific Northwest and Intermountain West) harvested 24.3 million kilograms of clean seed on ≈40,000 ha with a market value of $68 million (unpublished data from the Northwest Alfalfa Seed Growers Assoc.).

Alfalfa is largely self-fertile, but for mechanical reasons, flowers require bee visitation for pollination (reviewed in Bohart 1957, Free 1993, Richards 1996). Alfalfa flowers resemble small pea flowers, with a sexual column of fused stamens and pistil held under tension within the keel. When a visiting bee inadvertently "trips" the flower, the sexual column snaps upward, sometimes striking the bee. The sexual column is inevitably driven against the banner petal, which furls around it in <1 h. Once tripped, the flower can no longer be pollinated.

Commercial pollination of alfalfa is distinctive for an additional reason: both in Europe and the United States, most of the seed is produced using bees other than the honey bee, *Apis mellifera* L. Honey bees are used to some extent for producing seed of nondormant varieties of alfalfa in the Central Valley of California. The irrigation methods used there, plus the intense summer heat, allows growers to subject plants to repeated bouts of water stress, which reportedly diminishes the force of the floral tripping mechanism. Elsewhere, the honey bee has been shown to rarely trip the alfalfa flowers that it visits (Tysdal 1940, Reinhardt 1952, Bohart 1957, Dylewska et al. 1970). In North America, most seed alfalfa is pollinated by one of two managed nonsocial bee species. The alfalfa leafcutting bee, *Megachile rotundata* F., is a cavity-nesting Eurasian species first found in the United States in the 1930s (Stephen and Torchio 1961). The alkali bee, *Nomia melanderi* Cockerell, an alfalfa pollinator native to the United States (Intermountain West), is the only intensively managed ground-nesting bee in the world (Johansen et al. 1978). A number of additional nonsocial bee species around the world have been investigated and in a few cases locally used for their pollination qualities and manageability in alfalfa seed production (reviewed in Bohart 1957), but none has received widespread commercialization.

In comparing bees as alfalfa pollinators, studies have measured rates of floral visitation and proportions of visited flowers that are tripped (Dylewska et al. 1970); however, tripped flowers can differ in the likelihood of resultant pod set and seed content. In particular, selfed alfalfa flowers are more likely to abort their seeds than cross-pollinated flowers (Cooper and Brink 1940), setting fewer seeds per pod (Strickler 1999) and fewer pods (Free 1993). Bee species that pollinate other forage legumes differ in their abilities to set pods or seeds (reviewed in Richards 1996), possibly because they differ in their propensities to move pollen between conspecific plants and thus effect outcrossing. The purpose of this study was to compare the pollination efficiencies of managed North American alfalfa pollinators, plus two candidate pollinators of the genus *Osmia*, in terms of their floral tripping frequencies, likelihood of pod set, and their average yields of alfalfa seed per pod following single visits to virgin flowers on plants growing in a greenhouse.

Materials and Methods

In 1999, three varieties of alfalfa were seeded in alternating rows in the silt-loam floor of an unheated, evaporatively cooled greenhouse (8 by 15 m). 'Ranger' is an old public cultivar; 'Robust' and 'CG9802' are proprietary cultivars (ABI Alfalfa, Nampa ID). Rows were drip-irrigated to maintain soil moisture potentials of 22–36 centibars. Before bloom, rows were individually covered with light-weight spun-bonded polypropylene floating row covers that were supported above the canopy (1 m high) on strings and sealed at the floor. Two rows at one end of the greenhouse remained uncovered to maintain bees between experiments.

Five species of bee were chosen for release and comparison in the greenhouse in the summers of 1999 and 2000. Three species are commercial alfalfa pollinators: alfalfa leafcutting bee, alkali bee, and honey bee. Two cavity-nesting species considered as prospective alfalfa pollinators were also studied. These were *Osmia sanrafaelae* Parker, a native to the San Rafael Desert of Utah (Parker 1986), and a congeneric bee, *O. aglaia* Sandhouse, which was been found nesting amid concurrently managed populations of alfalfa leafcutting bees in Oregon, suggesting that it, too, might visit and pollinate alfalfa flowers.

Bees other than honey bees were overwintered at 4°C for 6 mo, incubated at 27°C, and released as adults in the greenhouse. A small barrel of soil, subirrigated and with a salt surface, was provided for nesting by alkali bees. Female *Osmia* spp. and *M. rotundata* were provided with drilled wooden nesting blocks. Honey bees were supplied in the greenhouse using a small nucleus colony consisting of four frames, several thousand workers, brood, and a laying queen. All bees were introduced and evaluated concurrently.

Once bloom had commenced, one or two sufficiently short sections (up to 3 m) of row cover were pulled back from the alfalfa for 1–3 h to allow one to four foraging bees access to the accumulated virgin flowers at a time. Observers followed bees as they landed on flowering racemes. Flowers visited once by a bee were scored as tripped or untripped during individual visitation bouts to racemes, and the species and sex of visitor noted. Following this first visit to a raceme, the entire raceme was immediately bagged in fine-mesh netting and given a numbered tag. A section of opened row was used only once. Bags were removed once all flowers had senesced. Resulting mature pods were counted, harvested, and their content of dark, plump seeds counted. Means ± 1 standard deviation are reported in the text.

Two preliminary analyses preceded statistical comparisons of bee species. First, it was desirable to pool all bee data for both years to maximize sample sizes. Because female *M. rotundata* provided the most visits in the 2 yr of experiment, the results of their visits were compared between years for (1) the proportions of visited flowers that they tripped (by categorical analysis, see below), (2) pod set, and (3) seed counts (both by analysis of variance [ANOVA], see below). Second, it was desirable to compare the proportions of visited flowers tripped per raceme without regard to the actual number of flowers that were visited. To justify this approach, data for female *N. melanderi* and *M. rotundata* were pooled to determine if the likelihood of tripping flowers on a raceme increased or decreased as a bee continued to visit more flowers on a raceme. For this analysis, data for tripping were grouped by the number of flowers visited per raceme (1, 2, 3 . . . flowers), and these groups compared by logistic regression for differences in tripping frequency.

Additional tripping frequency data come from field observations in a commercial seed field near Touchet, WA, at midday during peak bloom, June 25–26 2001. Foraging individuals of both sexes of alkali bees as well as honey bees were followed as they visited a series of 3–15 flowers. The proportions of flowers they were seen to trip were counted. Visits were pooled by species and sex.

Bee species were compared for tripping frequencies using log-linear models for categorical data (Proc CATMOD; SAS Institute 1989), beginning with the overall data sets (field and greenhouse), and then to contrast specific subsets. Overall log-likelihood ratio tests such as these can be partitioned into separate contrasts of subset classes in the data without inflating α levels (Sokal and Rohlf 1995). The proportion of tripped flowers on a visited raceme that yielded mature pods ("pod set") was calculated and log-transformed. Homogeneity of variances were checked by Levene's test (acceptable *P* values >0.05). Pod set per raceme was then weighted by the number of flowers that a bee visited on a raceme, reasoning that average pod set per raceme will be more reliably estimated if based on more visited flowers. One-way ANOVA (Proc GLM; SAS Institute 1989) was used to compare bee species for differences in their probabilities of pod set during floral visits and the numbers of seeds per pod that formed from tripped flowers. If significant, this was followed by Ryan-Einot-Gabriel-Welch pair-
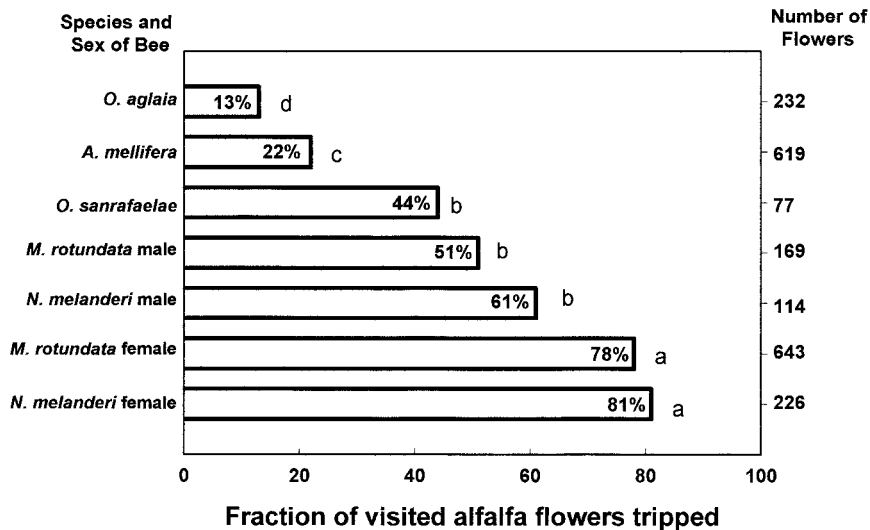
Fig. 1. Proportions of visited virgin alfalfa flowers that were tripped by visiting bee species and sexes flying in a greenhouse. Combinations of species and sex with different letters are significantly different by categorical analyses. The total number of flowers visited by each combination of species and sex is given in the right-hand column.

wise a posteriori comparisons. Species and sex of the two most common visitors, *N. melanderi* and *M. rotundata*, were compared by two-way ANOVA for tripping frequencies, pod set, and seed set.

Individual foraging females can be expected to visit several thousand alfalfa flowers per day, well in excess of the number of flower visits we were able to accumulate for each species in these greenhouse experiments. Therefore, for select comparisons between species of bees, minimum sample sizes were calculated that would be needed to detect significant differences in the proportions of flowers tripped, pods set, or the average seed set per pod (Sokal and Rohlf 1995). Given the actual means and variances found for the greenhouse data, $P = 0.05$ and an 80% certainty of being correct were chosen for the calculated estimate of minimum sample size needed to detect a significant difference between select species of pollinator.

Results

None of the bee species flown in the greenhouse nested avidly. Both *Osmia* species and *M. rotundata* were seen collecting leaf tissue for nesting; *M. rotundata* provisioned 54 nest cells and *O. sanrafaelae* provisioned 43 nest cells in the drilled nesting blocks. No alkali bee nested in the provided soil barrel, and none was seen carrying a sizeable pollen load while foraging.

Repeated opening and closing of the floating row covers resulted in very little inadvertent tripping of flowers (<1% of flowers tripped, $n = 500$). Self-tripping could also be indicative of uneven watering and periodic drought stress (Loper 1972). Soil moistures varied from 22–36 centibars during bloom in the greenhouse. This was apparently suitable to avert any noticeable water stress of the plants that might elicit

inadvertent self-tripping. The probabilities of tripping flowers did not change as a bee visited more flowers on an inflorescence (log-likelihood ratio $G = 0.04$, $P = 0.85$), allowing the analysis of tripping data by individual flower rather than by raceme or visitation bout.

Measured pollination attributes of female *M. rotundata* did not differ between the 1999 and 2000 greenhouse trials. The proportions of 585 visited flowers that were tripped during the 2 yr were equivalent ($G = 2.3$, $P = 0.13$, 82 versus 88%, respectively), as were the proportions of tripped flowers setting pods on racemes ($F = 0.88$; df $= 1$, 195; $P = 0.35$, 45 versus 54%). The average number of mature seeds set per pod by female *M. rotundata* was marginally different between years ($F = 3.90$; df $= 1$, 92; $P = 0.05$, 3.7 versus 6.9 seeds per pod), but was not different by a posteriori comparison. Data for the 2 yr of experiment were therefore pooled for subsequent analyses for all bee species.

Females of the five species of bees differed significantly in the proportions of the 2,080 visited alfalfa flowers in the greenhouse that they tripped ($G = 501$, $P <0.0001$) (Fig. 1). Female *O. sanrafaelae* tripped proportionally more visited flowers than honey bees ($G = 17.78$, $P <0.0001$) but the same proportion as male *M. rotundata* and male *N. melanderi* ($G = 5.75$, $P = 0.06$). Female *O. aglaia* tripped proportionally fewer flowers than honey bees ($G = 5.81$, $P = 0.016$). In the two-way ANOVA of species and sex, the respective sexes of *M. rotundata* and *N. melanderi* were equivalent in the proportions of the 1,152 visited flowers that they tripped ($G = 3.56$, $P = 0.06$), but males of these species were inferior to conspecific females in tripping flowers ($G = 50.7$, $P <0.0001$).

In the field, differences in tripping frequencies between honey bees and *N. melanderi* females and males were ordered the same as in the greenhouse experiments, but were more pronounced ($G = 107$, P

<0.0001). Thus, 22 female *N. melanderi* tripped 176 of 193 flowers visited (92%), 30 male *N. melanderi* tripped 49 of 119 flowers visited (41%), and 9 *A. mellifera* tripped 2 of 83 flowers visited (3%). As in the greenhouse, female alkali bees tripped proportionately more visited flowers than males ($G = 73$, $P <0.0001$), and male alkali bees tripped flowers more frequently than did honey bees ($G = 20$, $P <0.0001$). Tripping frequencies of alkali bees in the greenhouse and field settings were different from one another ($G = 17$, $P <0.0001$).

In the greenhouse, the species and sex of a floral visitor did not affect the likelihood of pod and seed set once a flower had been tripped. The proportion of tripped flowers producing pods averaged 48% and did not differ with the species of visiting female bee ($F = 0.79$; df = 4, 296; $P = 0.53$). Pods averaged 2.7 ± 2.1 mature seeds, which did not differ significantly among species of female floral visitor ($F = 1.15$; df = 4, 175; $P = 0.33$). Flowers tripped by male or female *N. melanderi* or *M. rotundata* were equally likely to yield pods ($F = 0.35$; df = 2, 261; $P = 0.70$, avg. 40–57% of tripped flowers) that did not differ in their counts of seeds ($F = 1.04$; df = 2,154; $P = 0.35$, avg. 2.2–3.2 seeds).

An estimated 405 floral visits would have been needed to detect a difference in the proportion of visited flowers tripped by male *N. melanderi* and *M. rotundata*. At least 4,100 floral visits would be required to detect differences in seed set between females of *N. melanderi* and *M. rotundata*. Superiority in seed set could be shown between either of these two species and the honey bee with just 280 single floral visits per species.

Discussion

As has been reported for just tripping rates by some of these same bee species (Bohart 1957, Dylewska et al. 1970, Batra 1976), species and sometimes sexes of bees in this study differed dramatically in their abilities to pollinate alfalfa flowers. In the greenhouse setting, the measured pollination differences between bees was entirely attributable to the likelihood that they would trip the sexual column of a visited flower. Flowers tripped by different species or sexes of bees were equally likely to yield pods containing similar numbers of seeds.

In this greenhouse experiment, the rank order and relative differences among bee species in tripping visited alfalfa flowers seemed applicable to the field, given the use of growing plants (rather than cut bouquets) of commercial varieties being visited by freely foraging bees in a uniform environment. However, females of *N. melanderi* tripped 92% of visited flowers in the field but only 81% in the greenhouse. Conversely, male alkali bees tripped proportionately more flowers in the greenhouse (61%) than in the field (41%). Female *M. rotundata* tripped 83% of visited alfalfa flowers in an extensive field study of commercial seed alfalfa near Nyssa, OR (Kemp and Bosch, unpublished data), a value in close agreement with the present greenhouse study (78%). These differences could be the result of environmental conditions imposed by the greenhouse, particularly the warmer, moister air, which influences the tripping mechanism, as does soil moisture in which the alfalfa is grown (Tysdal 1946, Lesins 1950, Loper 1972). Bees flying in the confines of the greenhouse probably had curtailed foraging flights as well, which could have altered their accumulation and transfer of pollen.

With other flowering plants, the floral resource sought by a foraging bee may influence its value as a pollinator. In particular, pollen-foraging individuals can be more effective pollinators than mere nectar foragers (Bader and Anderson 1962, Cane and Schiffhauer 2001) unless they are less likely to contact floral stigmas than nectar foragers (Wilson and Thomson 1991). Pollen-foragers are reported to trip a greater proportion of alfalfa flowers in one study (Batra 1976), but these results are difficult to interpret, owing to the use of cut bouquets of flowering alfalfa and the absence of statistical comparisons. In the current study, most visitors to the greenhouse alfalfa lacked noticeable scopal pollen loads, suggesting that they primarily sought only nectar. Alfalfa is peculiar, however, for a bee's apparent lack of influence over pollen removal. In the field, 15 flowers visited once by pollen-foraging female *N. melanderi* retain as much pollen (mean = 2,100 grains) as 15 manually tripped flowers (mean = 2,300 grains) (J. H. C., unpublished data), indicating that pollen harvest by bees is merely passive. It may be argued therefore that the nectar foragers in this study, none of which side-worked the flowers, should be representative of pollen foragers in terms of their effectiveness as alfalfa pollinators. This hypothesis deserves future analysis.

The contribution of male bees to pollination is often overlooked, but can be significant on the basis of individual floral visits (Motten et al. 1981, Parker and Frohlich 1983, Herrera 1987, Neff and Simpson 1990). Orchids in particular may rely on deceived male bees or wasps for their pollination (Kullenberg and Bergström 1976, Williams and Whitten 1983). Male *N. melanderi* and *M. rotundata* were equal or superior in tripping frequency to females of other bee species used in this study (Fig. 1) and were equivalent to their conspecific females in resulting pod and seed production at flowers that they tripped. Even in the field, male *Nomia* tripped 41% of virgin alfalfa flowers that they visited. Males need visit only enough flowers to fuel their flight, however, and may live for fewer days than females. They therefore can be expected to visit far fewer flowers during their lifetimes than females, as females must in addition visit enough flowers to obtain pollen and nectar to provision 10–20 nest cells (Hobbs 1956, Johansen et al. 1978). The spatial patterning of male foraging and their disturbance of foraging females at flowers may influence their contribution to alfalfa outcrossing, but these assertions are untested. Lifetime pollination values of males should not be overlooked, but they will probably be of less importance than conspecific females in all but a few cases.

The tripping rates of honey bees in the greenhouse environment likely represented a maximum value. The typically poor pollination performance of honey bees in commercial alfalfa seed fields has been known for decades; they often trip <5% of the flowers that they visit (Tysdal 1940, Bohart 1957, Dylewska et al. 1970), a figure affirmed by the 2.4% tripping rate reported here. Rather than be struck by a tripped sexual column while probing for nectar, a honey bee forager works flowers from the side, a behavior that fails to trip the sexual column and fails to pollinate the flower. This behavior is learned over the course of several days (Reinhardt 1952). Foragers from the greenhouse colony had no prior experience working alfalfa flowers and had no alternative floral resource available. In the greenhouse, only a few of them were seen working alfalfa flowers from the side. Conversely, in the field, nearly all observed *Apis* worked alfalfa flowers from the side, hence the infrequent tripping.

The two species of *Osmia* evaluated herein contrasted in their abilities to trip alfalfa flowers. Females of both species positioned themselves squarely on the flowers, much like *M. rotundata* or *N. melanderi*. However, from this position, *O. aglaia* was able to probe an alfalfa flower's nectaries using its tongue, without having to force its head between the banner petal and keel. The apparent greater length of this bee's tongue thus seems responsible for its infrequent floral tripping and so poor performance as an alfalfa pollinator. Bumble bee species with longer tongues trip far fewer alfalfa flowers than shorter-tongued species, but the mechanism is different. Long-tongued bumble bees learn to rob nectar by probing along the sides of the flowers, as do experienced honey bees (Gurr 1974 cited in Richards 1996).

Overall, 48% of tripped flowers set pods as a result of a bee visit in this greenhouse study. Bee species and sexes did not differ in the proportions of tripped flowers that later set pods. In a more carefully controlled greenhouse study using manually cross-pollinated flowers, Strickler (1999) likewise found that only half of tripped flowers ultimately yielded pods, well within the range of 40–57% average pod set observed in the current study for males and females of *M. rotundata* and *N. melanderi*. Evidently, the approximate 50% likelihood of a mature pod forming from a tripped flower is an upper limit imposed by the plant and is little influenced by the species or sex of pollinator.

Bee species and sexes likewise did not affect the number of seeds matured per pod at tripped flowers. The overall average of 2.7 seeds per pod resulting from bee visits to flowers exceeds that known for autopollination of alfalfa (e.g., less than 1 seed per pod) (Free 1993, Strickler 1999). Cross-pollination of alfalfa has been shown to yield more seeds per pod than geitonogamous pollination (Tysdal 1940, Lesins 1950, Pankiw et al. 1956, Bohart 1957, Strickler 1999), with honey bee pollination yielding an intermediate value (Pankiw et al. 1956). However, the reported differences and maximum values for seeds per pod are highly variable, probably due to a combination of factors that includes growing conditions, weather, cul-

tivar, available bloom, and the health, age and genotype of individual plants (Pedersen and Nye 1962, Strickler 1999). No cultivar differences were detected in this study. The average count of seeds per pod produced by bee-tripped flowers in the current study is equivalent to that recently reported (Strickler 1999) for manually pollinated greenhouse plants experiencing limited geitonogamy (mean = 2.2 seeds per pod) or pure outcrossing (mean = 3.3 seeds per pod). In the field component of Strickler's study, seed set per pod exceeded that of her greenhouse experiments for manual outcrossing. However, after a sequence of 5–10 sequential geitonogamous manual pollinations, seed set per pod in Strickler's field study was equivalent to the value found in this study following floral tripping by bees.

The average counts of mature seeds per pod were equivalent for all bee species with the modest sample sizes used in this study, but biologically meaningful differences may nonetheless exist between the bee species. Given the means and variances for seeds per pod, the inferiority of honey bees compared with female alkali bees or alfalfa leafcutting bees could be shown with <300 pods set per bee species, or ≈600 tripped virgin flowers. That is a daunting sample size to achieve using single visits in a greenhouse, but in the field, individual females can be expected to visit and trip that many flowers every hour of foraging (Bohart, 1957, Richards 1996). Hence, flowers tripped by females of the alkali bee and the alfalfa leafcutting bee can be expected to set more seed than those tripped by honey bees on a daily basis, but the primary difference between these bee species remains their floral tripping rates.

The greenhouse protocol for unveiling one or two partial rows of virgin flowers of one or at most two varieties probably favored geitonogamy, perhaps comparable to peak bloom in commercial fields that are typically blocks of a single variety. Modestly greater counts of seeds per pod than these have been reported for bee pollination of alfalfa in the field (Hobbs 1956). That study unfortunately lacked information about pod set; conceivably, pods with fewer fertilized ovules may abort under conditions of competition with seedier developing pods when maternal resources are limited, such that only pods with larger counts of seeds mature (reviewed in Stephenson 1981). In our greenhouse study, no plant had >50 pollinated flowers, so that all forming pods probably experienced adequate maternal resources for development. Ideally, alfalfa pollinators should be compared for floral visitation rates, tripping frequencies, pod set, and seed set under controlled field conditions, but that combination has thus far eluded experimental protocols despite over a half century of investigation.

Bees contribute to alfalfa pollination such that commercially acceptable production is no longer possible without effective managed pollinators. Candidate pollinators can differ dramatically in the proportion of visited flowers that they trip. Differences in tripping frequencies of bee species overshadow their differences in likelihood of pod formation or seed set per

pod, at least in these greenhouse experiments. Additional experiments will be needed to discern whether alfalfa pollinators differ in their propensities to cross-pollinate or self-pollinate alfalfa in the field.

Acknowledgments

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